

Ex-6

THE LEUCOCYTE ANTIGEN *FactsBook*

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Contents

Preface	VII
Abbreviations	VIII
Dedication	IX

Section I THE INTRODUCTORY CHAPTERS

<i>Chapter 1</i>	
Introduction	2
<i>Chapter 2</i>	
The Analysis and Architecture of the Leucocyte Cell Surface	13
<i>Chapter 3</i>	
Protein Superfamilies and Cell Surface Molecules	38
<i>Chapter 4</i>	
Chromosomal Organization of the Genes Encoding Leucocyte Surface Antigens	88

Section II THE LEUCOCYTE ANTIGENS

CD molecules					
CD1	102	CD27	160	CD53	222
CD2	104	CD28	162	CD54	224
CD3/TcR	106	CD29	164	CD55	226
CD4	110	CD30	166	CD56	228
CD5	112	CD31	168	CD57	231
CD6	114	CDw32	170	CD58	232
CD7	116	CD33	174	CD59	234
CD8	118	CD34	176	CDw60	237
CD9	120	CD35	178	CD61	238
CD10	122	CD36	182	CD62	240
CD11	124	CD37	184	CD63	242
CD12	129	CD38	185	CD64	244
CD13	130	CD39	187	CDw65	247
CD14	132	CD40	188	CD66	248
CD15	135	CD41	190	CD67	250
CD16	136	CD42a,b	192	CD68	252
CDw17	139	CD43	196	CD69	254
CD18	140	CD44	198	CDw70	257
CD19	142	CD45	202	CD71	258
CD20	144	CD46	206	CD72	260
CD21	146	CD47	209	CD73	263
CD22	148	CD48	210	CD74	264
CD23	150	CD49	212	CDw75	266
CD24	152	CDw50	217	CD76	267
CD25	154	CD51	218	CD77	268
CD26	158	CDw52	220	CDw78	269

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Molecular weights

Polypeptide 48400

SDS PAGE

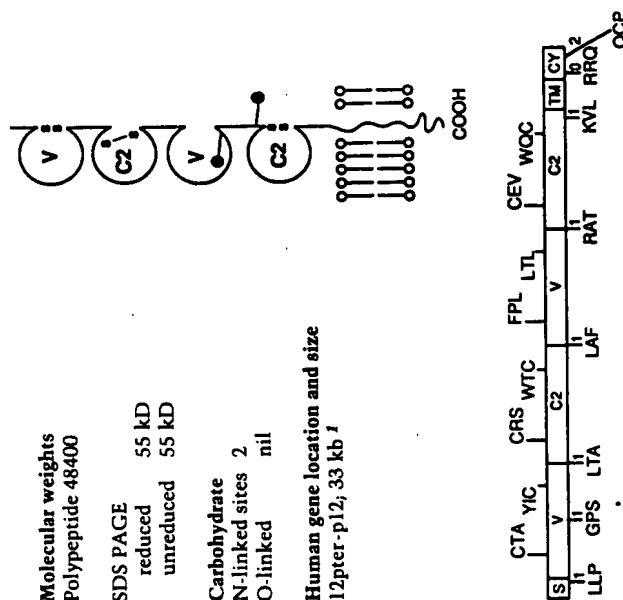
reduced 55 kD

unreduced 55 kD

Carbohydrate

N-linked sites 2

O-linked nil

Human gene location and size12pter-p12, 33 kb¹**Tissue distribution**

CD4 is expressed on most thymocytes and approximately two thirds of peripheral blood T cells, which constitute the CD8 negative cells². In human and rat but not in mouse, CD4 is expressed on monocytes and macrophages².

Structure

The extracellular domain is made up of four IgSF domains. The structures of the amino terminal two domains have been determined by X-ray crystallography, confirming that they are Ig-like^{3,4}. Domain 2 is characterized by an unusual disulphide within one beta sheet and domain 3 lacks a disulphide in the position conserved in most IgSF domains. Cat CD4 shows some unusual features with 17 residues inserted between domains 1 and 2⁵. There is an additional Cys in domain 1 and the Cys in the unusual β strand C position in domain 2 is replaced with a Trp and there is an extra Cys in the β strand F⁵. The position of the NH₂-terminus has been established for the rat homologue⁶.

Function

CD4 is an accessory molecule in the recognition of foreign antigens in association with MHC Class II antigens by T cells². MAbs against CD4

inhibit T cell functions *in vivo* and *in vitro*². The cytoplasmic domain of CD4 is phosphorylated at Ser residues 408, 415, 431 (see below) when T cells are activated by antigen or phorbol esters⁷. The cytoplasmic domain interacts with a lymphocyte-specific tyrosine kinase called p56^{lck} through a motif shown below⁸. CD4 is a receptor for HIV-1 (AIDS virus) and the binding of the viral gp120 protein is to a region of the amino terminal domain^{3,4}.

Comments

CD4 shows particularly close similarities in overall structure to the LAG-3 protein [see page 342].

Motifs involved in CD4 function

p56^{lck} recognition site (underlined) and Ser residues phosphorylated (in bold)

RRQAE RMSOI KRLLEKKTC QCPHRFQKTC SPI (433)

Database accession numbers

	PIR	SWISSPROT	EMBL/GENBANK	REFERENCE
Human	A02109	P01730	M12807	2
Rat	A27449	P05540	M15768	6
Mouse	A02110	P06332	M13816	2

Amino acid sequence of human CD4

HNRGVPFRHL LVLQLALLP AAT06 -1
 KKVVLGKGD TVELTCTASQ KKSIOFHWN SNQIKILGNQ GSFLTQGPSK 50
 LMDRADSRSS LWQDGNFPLI IKNLKIEDSD TYICEVEDOK EEVQLLVFGL 100
 TANSOTHLLO GQSLTTLIES PPGSPSVQOC RSPRGKNIQG GKTLSVSQLE 150
 LQSGTWTCT VLQOKKVEF KIDIVLAFQ KASSIVYKKE GEQVEFFSPL 200
 AFTVEKLTGS GELWQAEARA SSSKSWITFD LKNKEVSXKR VTQDPKLMG 250
 KKLPLHLTL P QALPOYAGSG NLYLALEAKT GKLHDEVNLY VMRATQLOKM 300
 LTCVWGTSP PKMLSLKLE NKEAKVSKRE KAVWLNPEA GHWQCLLSDS 350
 GQVLESNIK VLPWTSTPQV PHALLVLGGV AGLLLFILG LFFCYRCRHR 400
 RRQAE RMSOI KRLLEKKTC QCPHRFQKTC SPI 433

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CD28

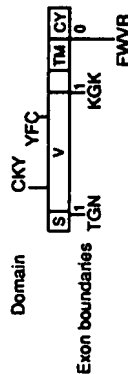
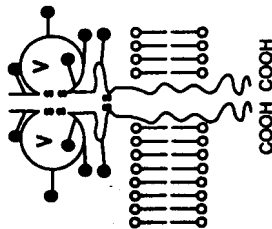
Tp44

Molecular weights
Polypeptide 23085

SDS PAGE
reduced 44 kD
unreduced 90 kD

Carbohydrate
N-linked sites 5
O-linked unknown

Human gene location and size
2q33-q34, 36 kb ¹



Tissue distribution

CD28 is expressed on most T lineage cells and plasma cells ². Mature thymocytes have higher levels of CD28 than the immature cells and among peripheral T cells, 95% of CD4⁺ cells and 50% of CD8⁺ cells are positive ². Activation of T cells leads to enhanced CD28 expression ².

Structure

CD28 is a member of the IgSF and is expressed as a disulphide-linked homodimer ^{2,3}. Human and mouse CD28 are 68% identical at the amino acid level ⁴. CD28 is particularly similar to CTLA-4 with which it shares a ligand and probably a common ancestor in evolution ⁴.

Function

The ligand for CD28 is B7 ^{5,6} which is expressed on activated B cells, suggesting an important role for CD28 in the interaction between T and B cells. Activation of T cells via CD28 has provided evidence for a CD28 signalling pathway which involves stabilization of cytokine mRNA levels and is separate from that used by the TcR-CD3 complex ^{2,7}.

Database accession numbers

PIR	SWISSPROT	EMBL/GENBANK	REFERENCE
Human	P10747	J02988	3
Mouse		M34563	4
Rat		X55288	8

Amino acid sequence of human CD28

MLRLLALNL FPSIQVTG
NKILVKQSPM LVAYDNAVNL SCKYSYNLFS REFRASLHKG LOSAVEVCVW
YGNYSQQLQV YSKTGFNCDG KLGHSVTFF LQNLVYVOTD IYFCKIEWMY
PPYLDNEKS NGTITHYKKG HLCPSPLFPG PSKPEWLVV VGGVLACYSL
LVIVAEILFH VRKRSRLH SDYNNMTPRR PGTRKKHYQP YAPPRDFAAY
RS

References

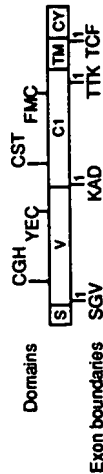
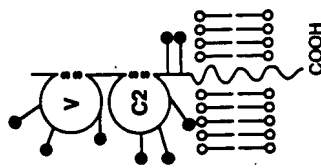
- 1 Lee, K.P. et al. (1990) J. Immunol. 145, 344-352.
- 2 June, C.H. et al. (1990) Immunol. Today 11, 211-216.
- 3 Aruffo, A. and Seed, B. (1987) Proc. Natl Acad. Sci. USA 84, 8573-8577.
- 4 Gross, J.A. et al. (1990) J. Immunol. 144, 3201-3210.
- 5 Linsley, P.S. et al. (1990) Proc. Natl Acad. Sci. USA 87, 5031-5035.
- 6 Linsley, P.S. et al. (1991) J. Exp. Med. 173, 721-730.
- 7 Lindsten, T. et al. (1989) Science 244, 339-42.
- 8 Clark, G.S. and Dallman, M.J. (1992) Immunogenetics 35, 54-57.

Molecular weights
Polypeptides 30048

SDS PAGE
reduced 60 kD
unreduced 60 kD

Carbohydrate
N-linked sites 8
O-linked unknown

Human gene location and size
3q13.3-3q21, 32 kb ¹



Tissue distribution

Present on a subset of B cells *in vivo* and the majority of B cells activated *in vitro*. Red blood cells, granulocytes, monocytes, resting or activated T cells, thymocytes and platelets do not express B7 ². The antigen is expressed by HTLV-1 transformed T cells ³.

Structure

The extracellular domain contains two IgSF domains which are highly glycosylated ⁴. The sequence of the transmembrane domain is unusual containing 3 cysteine residues that might be covalently modified or participate in intermolecular interactions ⁴ although there is no evidence for this. The cytoplasmic domain has a preponderance [9/19] of arginine residues and contains a potential site for calmodulin-dependent phosphorylation [RRES] ⁴.

Function

B7 is the ligand for the CD28 ⁵ and CTLA-4 ⁶ glycoproteins. Cells transfected with either human ⁷ or murine ⁸ B7 genes supply co-stimulatory signals to human T cells, suggesting that the CD28 binding site is conserved ⁸. The antigen is not expressed on resting B cells but is strongly upregulated on B cells activated with a variety of agents, including the Epstein-Barr virus ², cross-linking anti-IgM ², anti-CD45 and anti-MHC Class II mAbs ⁹, IL2 and IL4 ¹⁰. MAbs to B7 block the differentiation of B cells into antibody secreting cells ¹¹ and the alloactivation of T cells ⁹.

Comments

This antigen is not related to a mouse antigen called B7 and to avoid confusion the latter is being called B7(2).

Database accession numbers

Human M27533
Mouse X60958
PIR SWISSPROT EMBL/GENBANK REFERENCE
4 8

Amino acid sequence of human B7

MGHTRRQGTG PSKCPYLNNFF QLLVLA -1
GLSHFCSGVI HVTKEVKEVA TLSCGHNVSV EELAQTRIYW QKEKKHVLTM 50
MSGDMIMPE YKNTIFDIT NMLSVILAL RPSDEGTYES VVLKYEKDAF 100
KREHLAEVTL YKADFTPTS ISDFEIPSTN IRIICSTSG GFPEPHLSWL 150
ENGEELNAIN TTVSQDPETE LYAVSSGLDF NMTNHSFMC LIKYGHLRVN 200
QTFNNTTKQ EHPDNLPS MAILISYNG JEVICCLTYC EAPRCRRRR 250
NERLRRESVR PV 262

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- 6 Linsley, P.S. et al. (1991) J. Exp. Med. 174, 561-569.
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- 8 Freeman, G.J. et al. (1991) J. Exp. Med. 174, 625-631.
- 9 Koulova, L. et al. (1991) J. Exp. Med. 173, 759-762.
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